

# Irradiation as a possible quarantine treatment for green scale *Coccus viridis* (Green) (Homoptera: Coccidae)

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## Abstract

The green scale, *Coccus viridis* (Green), can be controlled effectively by irradiation at a minimum absorbed dose of 250 Gy. Reproductive capacity of irradiated gravid adults was reduced greatly and any resulting offspring were not able to develop beyond the crawler stage. Development of nymphs to the adult stage was not arrested completely nor was development of immature stages eliminated, but all survivors were sterile. Generally, higher doses of irradiation ( $\geq 400$  Gy) caused faster kill of all life stages than lower doses (250 Gy). At 250 Gy, there was prolonged survival of green scale, with 8.8–11.4% of nymphs and up to 8.8% of crawlers alive 3 months after irradiation; 100% mortality of the most resistant adult stage took longer than 20 weeks post-treatment. An absorbed dose of 500, 750 or 1000 Gy caused 100% mortality in all stages of the green scale by 7, 6, and 3 weeks post-treatment, respectively. Adults appeared to be more resistant to treatments  $\geq 500$  Gy. Irradiation doses  $\geq 500$  Gy killed crawlers by 3–5 weeks post-treatment and rendered nymphs and adult green scale sterile until their eventual death. When irradiated at 250 Gy, survival of non-infested gardenia plants and green scale-infested gardenia and coffee plants were reduced by 69, 56, and 18%, respectively, as compared with non-irradiated plants. Nonreversible, sublethal phytotoxicity included tip browning of young leaves, absence of new leaf growth in gardenia plants, failure to form new leaves in coffee plants, and eventual plant death. While irradiation at 250 Gy is sufficient to provide quarantine security for crops that are hosts of green scale, product quality will need to be evaluated on a case-by-case basis since certain commodities, such as gardenia seedlings, have sustained phytotoxic effects. © 2002 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

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In the past, irradiation research has focused primarily on the economically-important tephritid

flies (Balock et al., 1963; Seo et al., 1974). Results from such studies led to United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA, APHIS) approval of 250 Gy for disinfesting various fruits and vegetables for export from Hawaii (APHIS, 1996, 1998). While an adequate dose of irradiation causes irreversible damage to the reproductive and digestive tracts of treated insects, certain developmental stages are not killed immediately and may be alive for as long as non-irradiated controls (Goodwin and Wellham, 1990; Yathom et al., 1990; Dohino and Tanabe, 1993; Dohino et al., 1996). Rapid mortality may be achieved with higher irradiation doses; however, the probability of phytotoxicity of the host commodity will also increase with doses (Seaton and Joyce, 1992; Tanabe and Dohino, 1993, 1995).

Green scale, *Coccus viridis* (Green), is a major federal action quarantine pest in Hawaii and other tropical regions (APHIS, 1998) because of its limited distribution and wide host range (Deckle, 1976). The green scale is a significant pest to citrus (*Citrus* spp.), coffee (*Coffea arabica* L.), and gardenia (*Gardenia jasminoides* Ellis); however, its wide host range also includes rambutan (*Nephelium lappaceum* L.), litchi (*Litchi chinensis* Sonn.) (APHIS, 1996, 1998), cocoa (*Theobroma cacao* L.), guava (*Psidium guajava* L.), ixora (*Ixora* spp.), noni (*Morinda citrifolia* L.), natal plum (*Carissa macrocarpa* Eckl.), plumeria (*Plumeria rubra* L.) (Zimmerman, 1948), and hundreds of other ornamental, fruit and vegetable crops (Ben-Dov et al., 1999). Infested plants, especially young trees (less than 2 years after transplant), suffer stunting, yellowing and/or loss of leaves, and fruit drop (Mau and Kessing, 1999). A significant secondary effect of green scale infestations is the development of sooty mold on the honeydew excreted by the scale (Beardsley, 1952) that may interfere with the photosynthetic activity of the plant and has a negative impact on aesthetics (Deckle, 1976).

Irradiation trials specifically on green scale have not been reported, but a few studies have been conducted on other homopterous insects, such as greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Calvitti et al., 1997); beet

leafhopper, *Circulifer tenellus* Baker (Ameresekere and Georghiou, 1971); brown planthopper, *Nilaparvata lugens* Stahl (Mochida, 1973); and sugarcane delphacid, *Perkinsiella saccharidica* Kirkaldy (Osborn et al., 1966). Invariably, these studies showed that female sterility required lower doses of irradiation than male sterility (average 40 and 97.5 Gy for females and males, respectively, with males requiring 16.7–72.2% higher doses as compared with females). Populations of green scale are composed mainly of females (Hill, 1983) that are parthenogenetic and oviparous (Fredrick, 1943a,b); observations of males have not widely been documented (Hill, 1983). Theoretically, based on the data for the order Homoptera, a minimum of 25–50 Gy should control green scale with female sterility. However, many of the most economically important host plants for green scale are also hosts to fruit flies (e.g. citrus, coffee, rambutan, litchi, and guava); therefore, the irradiation dose approved by USDA, APHIS against tephritid fruit flies (250 Gy) will be the minimum dose required when these commodities are exported from Hawaii.

The objectives of this study were to (1) determine whether an irradiation dose of 250 Gy approved for tephritid fruit flies will also control the life stages of green scale; and (2) observe the effects of irradiation on two host plants of green scale, gardenia and coffee. Determining the effective irradiation dose needed to control the most resistant developmental stages of green scale will be used to provide support for approval of irradiation as a quarantine treatment for non-tephritid species by USDA, APHIS.

## 2. Material and methods

### 2.1. Mass rearing conditions

The original colony of green scale was collected from a natal plum hedge (*C. macrocarpa* Eckl.) at the University of Hawaii at Manoa, Honolulu, HI, and mass-reared in a laboratory on gardenia (*G. jasminoides* cv Veichii) plants

and coffee (*C. arabica* cv. Guatemalan) seedlings. Conditions in the lab holding cabinets were  $25 \pm 2$  °C average temperature, approximately 62–64% relative humidity due to room air-conditioning, and a 12:12 (L:D) photoperiod with light source provided by fluorescent plant lights (Model No. F40.PL, General Electric Co., Neal Park, Cleveland, OH). Gardenia plants were obtained from a local garden shop in 15.2-cm diameter pots and ranged in height from 12.5 to 25 cm; they were pruned to eight to ten leaves per plant. Coffee seedlings in 10.2-cm diameter pots were obtained from the University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources (CTAHR), Waiakea Agricultural Research Station, Hilo, HI, and ranged in height from 8 to 14 cm with four to six leaves per plant.

## 2.2. Dosimetry

All irradiation treatments were conducted at the University of Hawaii at Manoa, Hawaii Research Irradiator (HRI) in Honolulu, HI, which has a  $^{60}\text{Co}$  source of  $\gamma$ -radiation located 3.5 m below the surface of an 8516-l stainless-steel tank. The tank is filled with 18 °C deionized water serving as the shield from the radiation source. Dosage (Gy) was administered by placing samples inside an  $18 \times 53 \times 58$  cm treatment chamber that is lowered to the bottom of the tank via an electric hoist for a predetermined length of time based on the dose rate ( $6.8\text{--}5.5$  Gy  $\text{min}^{-1}$  during testing). A dose mapping of the area within the chamber where samples were placed was conducted to verify the accuracy and range of the doses applied. A total of 27 Gafchrome radiochromic film dosimeters (D-200, ISP Technologies, 1361 Alps Road, Wayne, NJ) that had previously been calibrated with alanine dosimeter standards quantified by the National Physical Laboratories' (Teddington, Middlesex TW11 0LW, UK) were positioned randomly on gardenia plants, natal plum cuttings, and in plastic specimen containers for the dosimetry study. Twenty-four hours after irradiation, films were read with a spectrophotometer (Perkin Elmer model 550) at 500-nm absorbance.

## 2.3. Irradiation trials

Two trials were conducted to determine survivability of the life stages of green scale when irradiated, (1) on gardenia and coffee plants; and (2) on a substrate and transferred to non-irradiated gardenia plants. A third trial was conducted to document the phytotoxic effects of irradiation on green scale-infested and non-infested host plants.

### 2.3.1. Irradiation of green scale on host plants

Gardenia and coffee plants were infested naturally with green scale when obtained and every stage of development was present at the time of treatment. On the gardenia plants, the average proportion of green scale present ( $n = 17\,268$ ) was 33.0% crawlers, 41.8% nymphs, and 25.2% adults. On the coffee plants, the average proportion of green scale present ( $n = 2690$ ) was 58.5% crawlers, 33.0% nymphs, and 8.5% adults.

There were 12 gardenia plants and eight coffee plants per treatment. Each potted plant was placed in a 7.6-l, 34.3- $\mu\text{m}$  thick plastic bag that was twisted shut to prevent accidental wetting from the irradiator's water shield, then irradiated. Gardenia plants were subjected to one of the four treatments: target doses 250, 500, 750, or 1000 Gy; coffee seedlings were subjected to one of the two treatments: target doses 250 or 500 Gy. In addition, 12 gardenia and eight coffee plants serving as controls (0 Gy) were also placed in bags and stored in an environmental chamber for the duration of the treatments at 18 °C to simulate average temperature in the irradiator. After treatment, each coffee and gardenia plant was removed from their plastic bag and placed on an inverted petri dish ( $1.5 \times 8.5$  cm diameter) set in a 24- or 29-cm-diameter plastic saucer, respectively, which contained tap water and four to five drops of dishwashing detergent to prevent crawlers from escaping. All plants were held at laboratory conditions described for mass-rearing.

Unhatched eggs and live and dead crawlers, nymphs and adult green scales were counted on each plant immediately after treatment and every 7 days thereafter to determine mortality. Crawlers were observed for the presence of two caudal

setae that are lost upon ecdysis. Change in shape and increase in size were criteria used to determine nymphal development into the adult stage. Fertile adults were identified by the presence of egg cast skins and newly hatched crawlers. Green scale behavior and activity were observed, including duration of crawler's search and preferred location on leaves for settling to feed. Resettling of nymphs and adults on leaves were also observed. Mortality of adults was determined by brown color with no observable movement. The duration of each developmental stage and the adults' ability to reproduce were also observed.

Percent survival data were transformed to arcsine (square root of the proportion) and analyzed by one-way ANOVA and Bonferroni's pairwise *t*-tests (Minitab, Minitab Inc., State College, PA, SigmaStat, Jandel Corp., San Rafael, CA). Linear regression analysis of the survival percentage for each life stage at each irradiation treatment over time after treatment was also performed (Minitab, SigmaStat).

### *2.3.2. Irradiated green scale transferred to non-irradiated plants*

Natal plum leaves naturally infested with green scale were obtained as described for the original colony (Section 2.1) and treated with target doses of 250, 400, 500, or 750 Gy. The average proportion of green scale present ( $n = 2586$ ) was 67.5% crawlers, 27.8% nymphs, and 4.7% adults. Immediately after irradiation, one to two leaves from the irradiated cuttings, depending on density of green scale infestation, were placed on the upper leaves of an uninfested gardenia plant and the insects were allowed to migrate there. There were 12 gardenia plants per treatment. Controls (0 Gy) were treated as described in the previous section. Counts of live and dead green scales and their stages of development on the gardenia plants were taken weekly. Statistical analyses (one-way ANOVA, Bonferroni's pairwise *t*-tests, and linear regression analysis) were done as previously described.

### *2.3.3. Irradiation of gardenia and coffee plants*

Gardenia and coffee plants already infested with green scale at different developmental stages

were obtained from a local nursery. Non-infested coffee plants were unavailable, so only infested coffee plants were irradiated at 250 and 500 Gy; there were four coffee plants per treatment. Infested and non-infested gardenia plants were irradiated at 250, 500, 750, and 1000 Gy; there were three gardenia plants per treatment. Each treatment was replicated four times. Four infested coffee plants and three infested and three non-infested gardenia plants serving as controls (0 Gy) were treated as described in the previous section. After irradiation, all plants were observed weekly for changes in leaves and stems, growth and development of new leaves, and length of survival after treatment. Observations were terminated 24 and 32 weeks after irradiation for coffee plants and gardenia plants, respectively. Longevity data were analyzed by one-way ANOVA and Bonferroni's pairwise *t*-tests.

## **3. Results**

### *3.1. Dosimetry*

Dosimetry was conducted at 250 Gy. The absorbed dose measurements ranged from 215 to 263. The maximum:minimum dose rate ratio was 1.22 in the irradiation chamber where samples were placed.

### *3.2. Irradiation trials*

#### *3.2.1. Irradiation of green scale on host plants*

The effects of irradiation on green scale infesting gardenia or coffee plants followed similar trends (Figs. 1 and 2). Among the controls, eggs were observed to hatch within minutes of being laid. As irradiation doses increased, the number of unhatched eggs observed 7-day post-treatment increased ( $P < 0.05$ ): 2.8, 14.4, 37.7, 60.8, and 66.9 unhatched eggs per adult at 0, 250, 500, 750, and 1000 Gy, respectively.

On gardenia plants (Fig. 1), at 250 Gy, some individuals of all stages survived beyond 7 weeks, but the host gardenia plants died 7–8 weeks after treatment, and subsequent observation of insects was terminated. When gravid adults were irradi-

ated at 250 Gy, new crawlers (17 crawlers from 80 adults, 0.21 offspring per adult) appeared within 1–7 weeks post-treatment, but they failed to develop and all died shortly after emergence. The adult and nymph stages survived beyond 7 weeks after being irradiated at 500 Gy, but all

crawlers died by 5 weeks post-treatment. At 750 Gy, 100% mortality was achieved at 5, 5 and 6 weeks post-treatment for crawlers, nymphs, and adults, respectively. At an absorbed dose of 1000 Gy, all stages were killed by 3 weeks post-treatment.

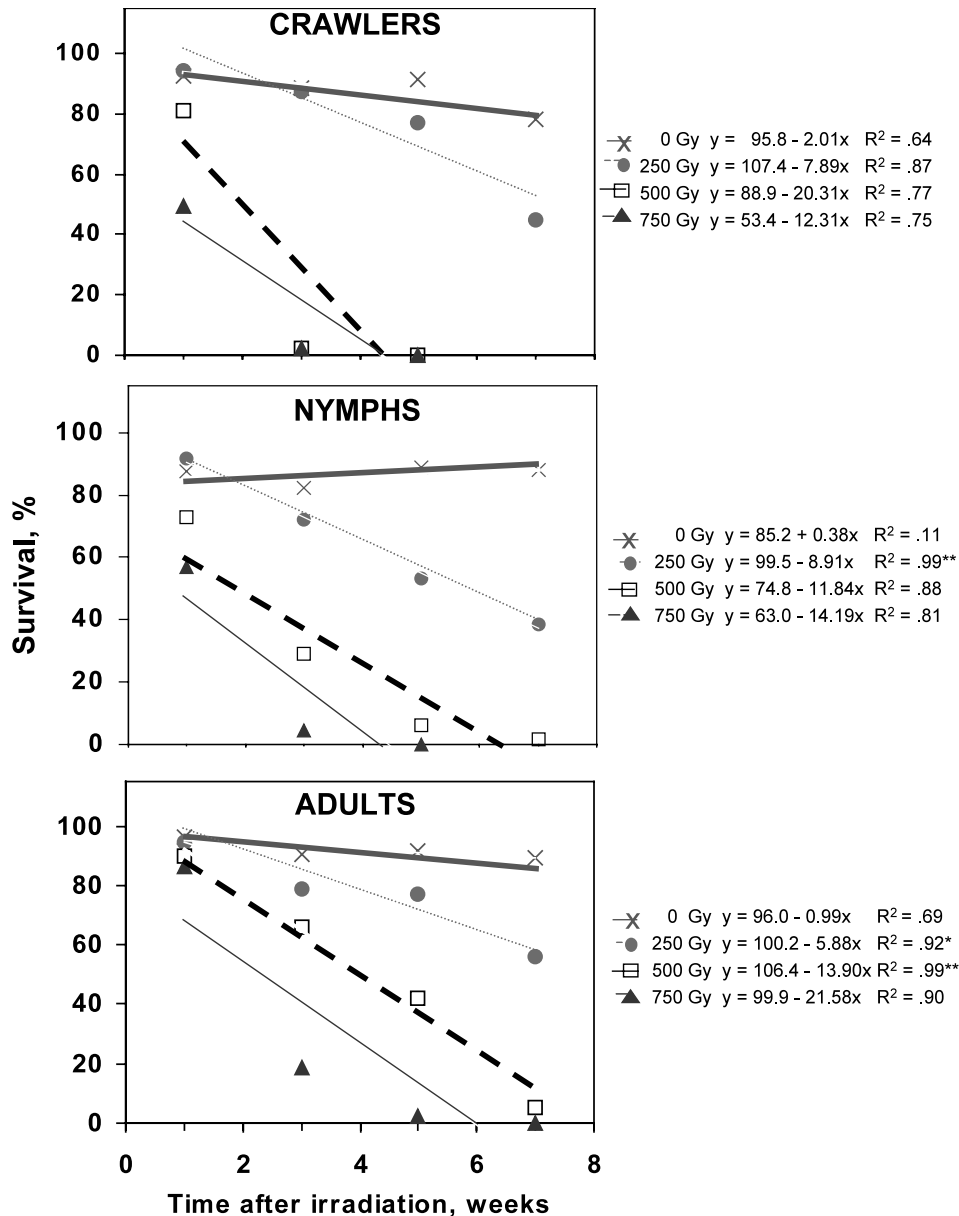


Fig. 1. Linear relationship between time after treatment vs. percentage survival for green scale nymphs, crawlers, and adults irradiated on gardenia plants (observation was suspended after 7 weeks; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).

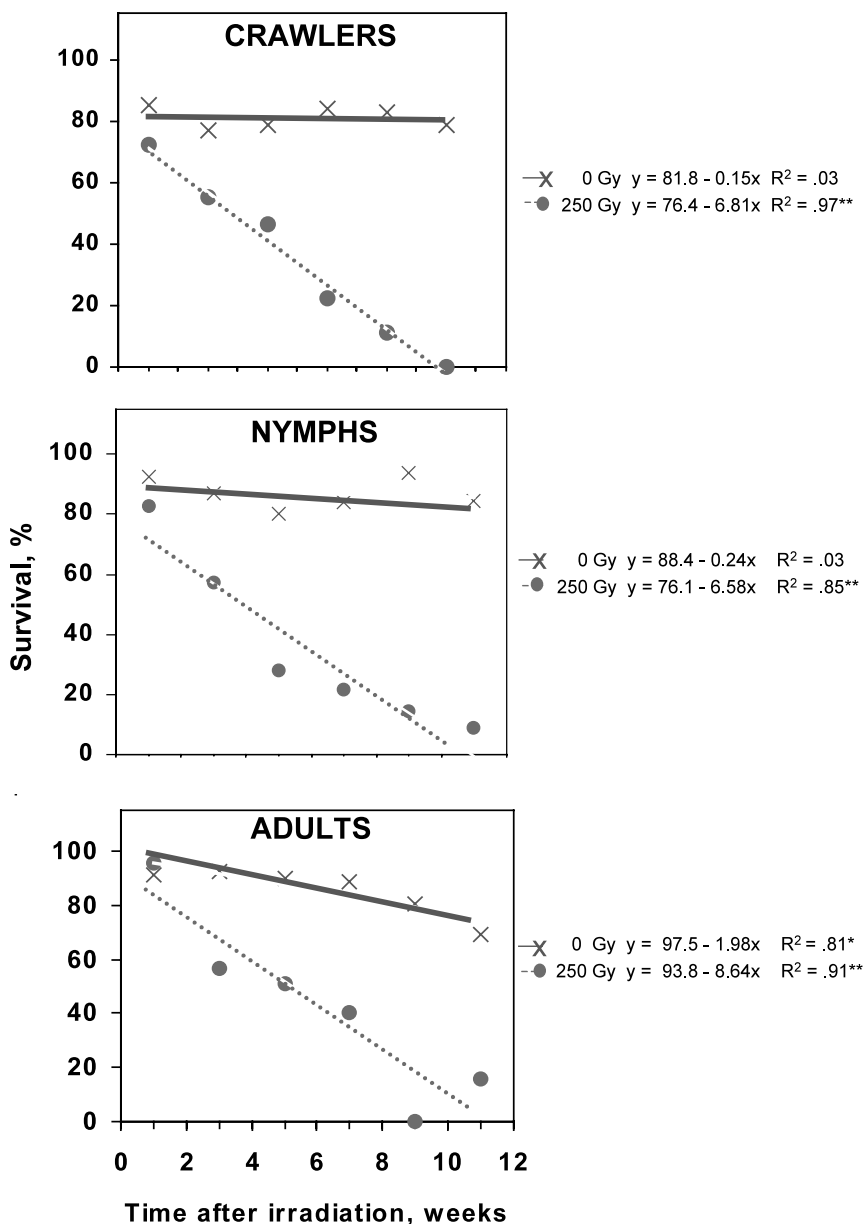


Fig. 2. Linear relationship between time after treatment vs. percentage survival for green scale nymphs, crawlers, and adults irradiated on coffee plants (observation was suspended after 12 weeks; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).

On coffee seedlings, all crawlers were dead by 13 and 3 weeks after treatment when irradiated at 250 and 500 Gy, respectively. All nymphs were dead by 5 weeks when irradiated at 500 Gy, but at 250 Gy, 0.50% (one out of 199 nymphs) survived up to 24 weeks, and all were sterile. All adults

were killed by 16 and 7 weeks when irradiated at 250 and 500 Gy, respectively. Reproduction in gravid adults irradiated at 250 Gy was reduced significantly but not prevented; however, their offspring were not able to develop beyond the crawler stage. It was difficult to maintain control

populations of green scale on coffee plants (Fig. 2), as evidenced by low survival percentages (78.6, 84.4, 69.4% at 12 weeks post-treatment for crawlers, nymphs and adults, respectively).

### 3.2.2. Irradiated green scale transferred to non-irradiated gardenia plants

Due to the susceptibility (shortened life) of the gardenia plants to irradiation and the difficulty of rearing green scale on coffee seedlings under laboratory conditions, green scale were irradiated on naturally-infested natal plum cuttings and transferred onto non-irradiated gardenia plants in order to observe the long-term effects of irradiation on the different life stages.

As the irradiation dose to which green scales were exposed increased, rate of their survival on non-irradiated gardenia plants decreased (Fig. 3).

At 250 Gy, 0.8% of the nymphs (one out of 130) and 0.3% of the crawlers (one out of 350) survived longer than 20 weeks, and 4.6% of the nymphs (six out of 130) developed into adults but none were able to reproduce. All adults were dead by 14 weeks after being irradiated at 250 Gy. There were no differences ( $P > 0.05$ ) in survival percentages between stages of development when irradiated at 250 Gy; however, adult green scale appeared to be more resistant to irradiation doses of  $\geq 400$  Gy as compared with crawlers and nymphs. Irradiation at 400 Gy caused 100% mortality in crawlers, nymphs, and adults by 6, 6 and 7 weeks, respectively. At either 500 or 750 Gy, 100% mortality of crawlers, nymphs and adults was observed at 3, 5, and 6 weeks, respectively.

### 3.2.3. Irradiation of gardenia and coffee plants

Ionizing radiation reduced ( $P < 0.05$ ) survival of all plant types as compared with untreated plants (Table 1). Nonreversible sublethal effects of ionizing radiation included tip browning of young leaves and absence of new leaf growth in gardenia plants and failure to form new leaves in coffee plants, followed by eventual plant death.

When irradiated, the non-infested and infested gardenia plants survived 20–35 and 24–44% as long as controls, respectively. Irradiated coffee plants survived 63–82% as long as controls; however, they were exposed only up to a dose of 500

Gy, the highest dose at which green scale-infested coffee plants were treated in the previous experiment.

Among the control gardenia plants, uninfested plants survived longer ( $P < 0.05$ ) than infested plants, but when irradiated at any dose, there was no difference ( $P > 0.05$ ) in length of survival.

Non-irradiated, infested coffee seedlings outlasted ( $P < 0.05$ ) both infested and non-infested gardenia controls. Infested coffee plants were more resistant to irradiation at 250 and 500 Gy, surviving longer ( $P < 0.05$ ) after treatment, as compared with either infested or non-infested gardenia plants.

## 4. Discussion

Attempts to observe the effects of irradiation on green scale infesting gardenia or coffee plants met with two unforeseen circumstances, (1) the host gardenia plants died shortly after 7 weeks post-treatment, therefore, subsequent observation of insects ceased, even though all life stages of green scale continued to survive; and (2) green scale did not fare well on coffee plants under laboratory conditions and mortality was high among controls. These findings necessitated transfer of green scale irradiated on naturally-infested natal plum cuttings onto non-irradiated host plants in order to observe survivability of all life stages. In addition, a trial was conducted to determine survival times of host plants either infested or non-infested after irradiation.

Comparing the effects of irradiation on green scale on the three host plants (irradiated on coffee, irradiated on gardenia, and irradiated on natal plum then transferred to non-irradiated gardenia), the same trends were evident. Generally, across all host plants, lower doses of irradiation (250 Gy) caused slower mortality of all life stages (smaller slopes (b)), while higher doses of irradiation ( $\geq 400$  Gy) caused faster kill. Regardless of host plant, as the irradiation dose increased, green scale survival rate tended to decrease (Figs. 1–3). Adults appeared to be more resistant to treatments  $\geq 500$  Gy. Irradiation doses  $\geq 500$  Gy prevented development of all stages, hindered re-

production of adult green scales and caused eventual death. Irradiation at 250 Gy did not completely eliminate development of immature stages or adult emergence from nymphs, but all were sterile.

In this study, the adult stage of green scale appeared to be more tolerant than the egg,

crawler or nymph stages. These results were in agreement with results of Dohino et al. (1996) on irradiation studies of *Thrips palmi* Karny and *Thrips tabaci* Lind. Younger eggs of the Comstock mealybug, *Pseudococcus comstocki* Kuwana, were more sensitive to irradiation than older eggs;

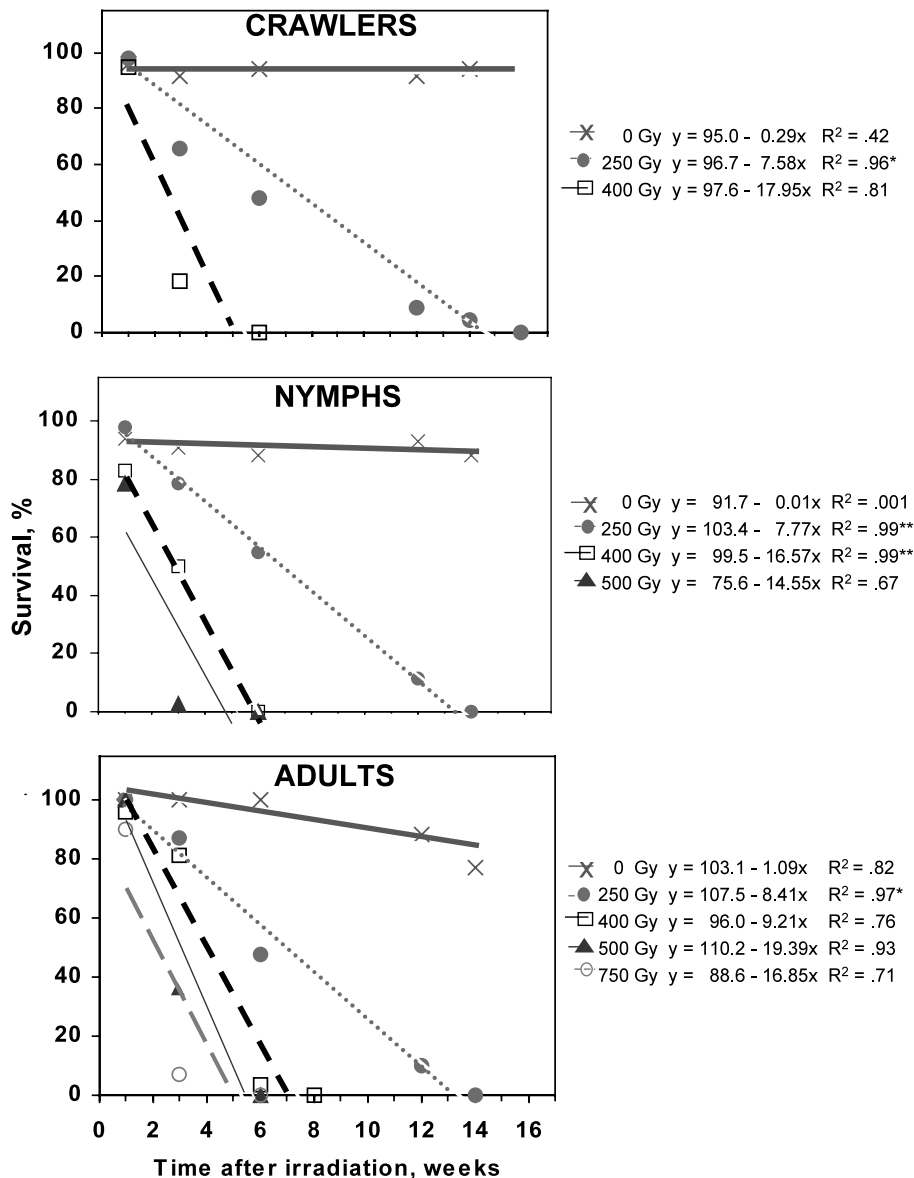


Fig. 3. Linear relationship between time after treatment vs. percentage survival for green scale nymphs, crawlers, and adults irradiated on natal plum cuttings and transferred to non-irradiated gardenia plants (observation was suspended after 16 weeks; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).



Table 1  
Survival of gardenia and coffee plants after treatment with  $\gamma$ -radiation

Dose (Gy)	Plant survival (weeks)		
	Non-infested Gardenia	Infested gardenia	Infested coffee
0	22.3 $\pm$ 0.8 <sup>a</sup>	14.3 $\pm$ 0.8 a	30.3 $\pm$ 0.6 a
250	7.0 $\pm$ 0.4 b	6.3 $\pm$ 0.5 b	24.9 $\pm$ 2.0 b
500	7.9 $\pm$ 0.8 b	5.7 $\pm$ 0.2 b	19.0 $\pm$ 0.8 c
750	6.4 $\pm$ 0.5 b	5.8 $\pm$ 0.3 bc	Not tested
1000	4.4 $\pm$ 0.7 b	3.4 $\pm$ 0.5 c	Not tested

Means in each column followed by different letters are significantly different ( $P < 0.05$ ).

<sup>a</sup> Standard error of means.

female adults irradiated at  $> 200$  Gy oviposited but their eggs failed to hatch (Dohino and Masaki, 1995). The differences in tolerance between insect stages may be explained by Bergonie and Tribondeau (1959), who stated that the degree of susceptibility to radiation is proportional to the degree of reproduction activity in cells and the level of differentiation of cells. Therefore, immature stages undergoing active cell division are more sensitive to radiation than the adult stage. This also applies to the growing tissue of the host plant, such as buds and growing tips.

Irradiation at a minimum absorbed dose of 250 Gy has been approved as a quarantine treatment for certain fruits and vegetables in Hawaii to control four species of fruit flies found in the state. This study has demonstrated that 250 Gy can prevent the establishment of new colonies of green scale; therefore, irradiation is a suitable quarantine treatment for agricultural products that are known hosts for green scale. The effects of irradiation on the treated commodity could be another limiting factor. Agricultural commodities that are sensitive to irradiation, such as gardenia plants, will require an alternative quarantine treatment.

After determining the minimum irradiation dose to control effectively the spread of specific quarantine pests, such as green scale, various host commodities need to be tested for their tolerance to that absorbed dose. Green scale are

polyphagous with many of their hosts being among the exportable commodity crops in Hawaii and other tropical regions, such as papaya, rambutan, litchi, citrus, cut flowers and foliage. This and other studies (Kader, 1986; Tanabe and Dohino, 1993, 1995; Chen and Paull, 1995; Moy et al., 1999) have shown that plants and plant parts, like insects, differ in their sensitivities to irradiation. In addition to variation in sensitivity among developmental stages of green scale to irradiation, duration of various stages in their life cycles have been shown to differ on gardenia and coffee plants, indicating host–pest specificity which must also be considered when determining the proper irradiation dose needed for quarantine security.

## 5. Conclusion

Irradiation at 250 Gy should be adequate as a quarantine treatment for green scale; however, the effects of irradiation will not be observed immediately after treatment and complete mortality may take longer than 20 weeks.

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